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Abstract

Desorption electrospray ionization-mass spectrometric (DESI-MS) analysis has been applied to the direct analysis of chemical warfare agents spiked onto a variety of sample media including soils, water, food products and indoor samples that could be collected during a forensic investigation following a chemical incident. Solid phase microextration (SPME) fibers were used in this investigation to sample the headspace above five organophosphorus chemical warfare agents, O-isopropyl methylphosphonofluoridate (sarin, GB), O-pinacolyl methylphosphonofluoridate (soman, GD), O-ethyl N,N-dimethylphosphoramidocyanidate (tabun, GA), O-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX). The exposed SPME fibers were introduced directly into a modified Z-spray electrospray (ESI) source, enabling rapid and safe DESI-MS analysis of the toxic chemical warfare agents. Time-aligned parallel (TAP) fragmentation data, which provides both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n, where n=2 or 3) data for an individual compound, were acquired for the first time for organophosphorus chemical warfare agents. Unique ion mobility profiles and up to six full scanning MS^n spectra, containing the $[M+H]^+$ ion and up to seven diagnostic product ions, were acquired for each chemical warfare agent during DESI-IMS-MS^n analysis of the exposed SPME fiber analysis. A rapid screening approach, based on the developed methodology, was applied to several typical forensic media (Dacron sampling swabs, office furniture fabric and cardboard) spiked with 5 µg of chemical warfare agent. Background interference was minimal and the spiked chemical warfare agents were readily identified within a minute on the basis of the acquired ion mobility and mass spectrometric data. Application of this approach is anticipated for high sample throughput scenarios requiring the increased levels of confirmation associated with the use of two spectrometric techniques.

Résumé

On a appliqué la méthode désorption - ionisation par électronébulisation - spectrométrie de masse (DESI-SM) pour l'analyse directe d'agents de guerre chimique dont on avait enrichi divers milieux d'échantillons, notamment de sol, d'eau, de denrées alimentaires et d'air intérieur, qui pourraient être prélevés dans le cadre d'une enquête judiciaire après un accident mettant en cause des agents chimiques. Au cours de cette étude, on a utilisé des fibres de microextration en phase solide (MEPS) pour échantillonner l'espace libre au-dessus de cinq agents chimiques organophosphorés, l'O- isopropylméthylphosphonofluoridate (sarin, GB), l'O-pinacolylméthylphosphonofluoridate (soman, GD), l'O-éthyl-N,N-diméthylphosphoramidocyanidate (tabun, GA), l'O- cyclohexylméthylphosphonofluoridate (cyclohexylsarin, GF) et l'O-éthyl-S-2- diisopropylaminoéthylméthylphosphonothiolate (VX). Les fibres de MEPS exposées ont été introduites directement dans une source modifiée d'électronébulisation de type ZSpray (ESI), qui permet une analyse DESI-SM rapide et sécuritaire des agents chimiques toxiques. Pour la première fois avec des agents de guerre chimique organophosphorés, on a obtenu des données de fragmentation parallèle alignées dans le temps (TAP), qui permettent d'obtenir des données de spectrométrie de mobilité ionique (SMI) et de spectroscopie de masse en tandem (SM^n, où n=2 ou 3) pour un composé individuel. On a obtenu des profils de mobilité ionique unique et jusqu'à six spectres de balayage MS^n, qui montraient l'ion [M+H]+ et jusqu'à sept ions de produits de diagnostic pour chaque agent de guerre chimique pendant l'analyse DESI-SMI-SM^n de l'analyse des fibres MEPS exposées. On a appliqué une approche de dépistage rapide, fondée sur la méthodologie ainsi développée, à plusieurs milieux d'analyse judiciaire typiques (écouvillons d'échantillonnage en dacron, tissu de mobilier de bureau et carton) enrichis avec 5 µg d'agent de guerre chimique. L'interférence due au bruit de fond était très faible et on a identifié rapidement, en moins d'une minute, les agents chimiques utilisés pour l'enrichissement grâce à ces données de mobilité ionique et de spectrométrie de masse. On prévoit des applications de cette approche pour des scénarios à fort débit d'échantillons, qui requièrent les niveaux accrus de confirmation associés à l'utilisation de ces deux techniques spectrométriques.

Executive summary

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) Analysis of Organophosphorus Chemical Warfare Agents: Rapid Acquistion of Time-Aligned Parallel (TAP) Fragmentation Data

Paul A. D'Agostino and Claude L. Chenier, DRDC Suffield TM 2010-047, Defence R&D Canada – Suffield, June 2010.

Introduction: Desorption electrospray ionization-mass spectrometric (DESI-MS) analysis has been applied to the direct analysis of chemical warfare agents spiked onto a variety of sample media including soils, water, food products and indoor samples that could be collected during a forensic investigation following a chemical incident. DESI-MS, first applied to chemical warfare agents at DRDC Suffield several years ago, allows rapid, direct sample analysis of solid-phase microextraction (SPME) fibers, the sampling media selected by Canada for sampling in the field. This approach has attracted considerable interest in the chemical defence and public security communities due to the minimal sample handling requirements and potential for rapid sample throughput.

Results: SPME fibers were used in this investigation to sample the headspace above five organophosphorus chemical warfare agents at DRDC Suffield. The exposed SPME fibers were introduced directly into the mass spectrometer and both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n, where n=2 or 3) data were acquired for the first time for organophosphorus chemical warfare agents. A rapid screening approach, based on the developed methodology, was applied to several typical forensic media (Dacron sampling swabs, office furniture fabric and cardboard) spiked with 5 μg of chemical warfare agent. Background interference was minimal and the spiked chemical warfare agents were readily identified within a minute.

Significance: Application of this approach is anticipated for high sample throughput scenarios requiring confirmation with two spectrometric techniques. Use of the developed methodology is anticipated during forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution or to assess remediation/restoration efforts following an incident.

Future plans: The reported method will be a valuable addition to the present methods for the identification of chemical warfare agents and their hydrolysis products in samples collected in support of counter-terrorism. Recent installation of the new Synapt HDMS^{TM} high resolution tandem mass spectrometer at DRDC Suffield will enable continued novel method development.

Sommaire

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) Analysis of Organophosphorus Chemical Warfare Agents: Rapid Acquistion of Time-Aligned Parallel (TAP) Fragmentation Data

Paul A. D'Agostino et Claude L. Chenier, DRDC Suffield TM 2010-047, R & D pour la défense Canada – Suffield; Juin 2010.

Introduction : On a appliqué la méthode désorption – ionisation par électronébulisation - spectrométrie de masse (DESI-SM) pour l'analyse directe d'agents de guerre chimique dont on avait enrichi divers milieux d'échantillons, notamment de sol, d'eau, de denrées alimentaires et d'air intérieur, qui pourraient être prélevés dans le cadre d'une enquête judiciaire après un accident mettant en cause des agents chimiques. La DESI-MS, appliquée pour la première fois à des agents de guerre chimique à la DRDC Suffield il y a plusieurs années, permet des analyses rapides et directes d'échantillons de fibres de microextration en phase solide (MEPS), le milieu d'échantillonnage sélectionné par le Canada pour l'échantillonnage sur place. Cette approche a suscité un intérêt considérable dans les milieux de la défense chimique et de la sécurité publique à cause de ses faibles exigences pour la manipulation des échantillons et de son potentiel d'échantillonnage rapide.

Résultats : Dans cette étude, on a utilisé des fibres MEPS pour échantillonner l'espace vide au-dessus de cinq agents chimiques organophosphorés à la RDDC Suffield. On a introduit directement les fibres MEPS exposées dans le spectromètre de masse et, pour la première fois avec des agents de guerre chimique organophosphorés, on a obtenu des données de spectrométrie de mobilité ionique (SMI) et de spectroscopie de masse en tandem (SM^n, où n=2 ou 3). On a appliqué une approche de dépistage rapide, fondée sur la méthodologie ainsi développée, à plusieurs milieux d'analyse judiciaire typiques (écouvillons d'échantillonnage en dacron, tissu de mobilier de bureau et carton) enrichis avec 5 µg d'agent de guerre chimique. L'interférence due au bruit de fond était très faible et on a identifié rapidement, en moins d'une minute, les agents chimiques utilisés pour l'enrichissement.

Importance : On prévoit des applications de cette approche pour des scénarios à fort débit d'échantillons, qui requièrent une confirmation à l'aide de ces deux techniques spectrométriques. On prévoit l'utilisation de ces nouvelles méthodologies dans le cadre des enquêtes judiciaires qui requièrent des données probantes indiquant l'utilisation d'agents de guerre chimique pour des poursuites au criminel ou pour l'évaluation des efforts d'assainissement ou de remise en état après un accident.

Plans pour l'avenir : Cette méthode est une technique utile qui complète les méthodes actuelles utilisées pour l'identification des agents chimiques et leurs produits d'hydrolyse dans des échantillons recueillis dans le cadre de la lutte antiterrorisme. L'installation récente du nouveau spectromètre de masse en tandem Synapt HDMS^{MC} à haute résolution à la RDDC Suffield devrait permettre de poursuivre le développement de cette nouvelle méthode.

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Introduction

The al-Qaeda terrorist attacks of 11 September 2001 heightened security concerns within many countries, resulting in the allocation of considerable resources for the development of improved field and laboratory based detection and identification methods for chemical warfare agents and other agents of concern. Analytical methods for the detection and identification of chemical warfare agents, their degradation products and related compounds has been thoroughly reviewed over the past decade with several different emphases [1-10]. Past analytical methods development was driven by the requirements of the military and their need to be able to detect and identify these compounds in typical battlefield samples [9]. Typical analyses involved extraction of the media of interest and analysis of the extracts for the presence of chemical warfare agents by gas chromatography-mass spectrometry (GC-MS) [1, 5, 6, 10] or liquid chromatography-mass spectrometry (LC-MS) [3, 7-10]. Increasingly, efforts are being made to determine the presence of chemical warfare agents, or their hydrolysis products, in biomedical [9] and forensic samples [10] that might be collected in support of public security concerns following a chemical event. A challenge associated with analyses for public security purposes is the likelihood of larger sample numbers. Higher throughput methods that still provide a similar level of certainty to the chromatography-based approaches would be beneficial.

Recently a novel mass spectrometric method for sample ionization and analysis, developed by Cooks' group and referred to as desorption electrospray ionization (DESI), was described [11]. During the DESI experiment charged droplets in the solvent being electrosprayed impact the surface of interest, desorbing and ionizing the analyte. Ionized large biomolecules and small organic molecules may then be detected by mass spectrometry, often in the tandem mode. Cooks recently reviewed ambient mass spectrometry with an emphasis on the DESI method [12] and included discussion on direct analysis in real time (DART) [13], a related direct analysis approach. DESI-MS is applicable to a range of compounds and offers the analyst a number of advantages that may be helpful for higher sample throughput. Advantages include rapid analysis (seconds to minutes), ease of sample introduction, little or no sample preparation, lack of sodium adducts and good sensitivity.

DESI-MS has been used for a variety of direct analyses [14], including the analysis of pharmaceutical products [15-26], dyes on thin layer chromatography plates [27], explosives on a variety of surfaces [22, 28-30], polymers [31], alkaloids on plant tissue [32], chemical warfare agents on solid phase microextraction (SPME) fibers [33, 34], hydrolysis products of chemical warfare agents on Teflon or glass surfaces [35] and pesticides on immobilized powders or various surfaces (glass, PTFE, Kapton and paper) [36]. Cooks first reported DESI-MS data for dimethyl methylphosphonate (DMMP) from a nitrile glove surface [11]. A protonated adduct of DMMP at m/z 124 (should be m/z 125) was observed. This assignment was corrected during subsequent investigations involving the DESI and/or desorption atmospheric pressure chemical ionization (DAP CI) analysis of DMMP from a variety of surfaces including paper [24, 29, 30], computer plastic and metal [30]. Similar results were also obtained during DESI-MS analysis of cloth and human skin spiked with DMMP [22].

Chemical warfare agents were first analysed by DESI-MS at DRDC Suffield from SPME fibers used to sample the headspace above neat chemical warfare agents and chemical warfare agents spiked onto Dacron sampling swabs and office media including office carpet, office furniture fabrics and photocopy paper [33, 34]. DESI-MS applications have been extended to chemical warfare agent hydrolysis products, including methyl phosphonic acid, isopropyl methylphosphonic acid and ethyl methylphosphonic acid, all of which were successfully analysed from Teflon surfaces [35]. A variant of DESI using boric acid, reactive DESI, was also employed to determine the presence of these hydrolysis products at nanogram levels in urine applied to a glass surface [35]. These hydrolysis products, as well as pinacolyl methylphosphonic acid and thiodiglycol, have also been successfully analysed during DESI-MS analysis of SPME fibers at DRDC Suffield.

Pawliszyn authored a recent text that describes in detail SPME sampling, including numerous methods and applications [37]. SPME has tremendous potential for the sampling of more volatile compounds, such as chemical warfare agents. Several methods have been reported in the literature with most of these being based on SPME sampling followed by GC-MS analysis [38-46]. Headspace sampling with SPME fibers has been reported for numerous chemicals and this type of sampling was envisioned to support the Canadian Forces. Samples collected in the field could be contained in a septum-sealed vial, the headspace above which may then be safely sampled using a SPME fiber [47] prior to GC-MS or DESI-MS analysis [33, 34].

Ion mobility spectrometry (IMS) has been used successfully for the detection of a variety of smaller molecules including explosives and chemical warfare agents, with the hand-held Chemical Agent Monitor (CAM) being one of the more recognized IMS devices [48]. The Synapt HDMS^{TM}, a tandem mass spectrometer containing an ion mobility cell in the Triwave^{TM} collision region between the quadrupole and time-of-flight mass analysers, has the potential to acquire both ion mobility and tandem mass spectrometric data during a single experiment. Applications with this relative new instrument have dealt largely with the analysis of larger biomolecules, even though IMS has historically been used largely for the analysis of smaller molecules [48]. Only one application of this unique instrument for the analysis of smaller molecules, the components of pharmaceutical formulations, was noted during literature review [26].

IMS has been interfaced to a variety of mass spectrometers, with Hill et al. reporting application of IMS-MS for the rapid separation and analysis of chemical warfare agent simulants and degradation products [49-54]. Most recently high field asymmetric waveform IMS-MS was utilized for the analysis of chemical warfare agents spiked into food products [55]. Incorporation of an IMS cell within the Triwave^{TM} collision cell of the Synapt HDMS^{TM} allows acquisition of both IMS data and MS^n (n=2 or 3) data for chemical warfare agents following DESI sample introduction. Data acquired in this manner, referred to as time-aligned parallel (TAP) fragmentation data, were evaluated for the rapid confirmation of chemical warfare agents.

This paper reports the first application of DESI-IMS-MS^n for the confirmation of chemical warfare agents. DESI-IMS-MS^n was investigated for the analysis of SPME fibers exposed to the headspace above five organophosphorus chemical warfare agents of concern to the defence and public security communities. Individual chemical warfare agents were differentiated on the basis of their IMS profiles and acquired full scanning, high resolution MS^n data. MS^n data contained

evidence of the $[M+H]^+$ ion and at least three other characteristic product ions. A screening approach was also developed for the analysis of sarin, soman, tabun and cyclohexyl sarin. Individual chemical warfare agents were also spiked onto Dacron sampling swabs, office furniture fabric or cardboard and screened for the presence of all four chemical warfare agents in a single DESI-IMS-MS^n analysis. Media blanks contained minimal chemical interferences and in all cases the spiked chemical warfare agent was readily detected. Full scanning, high resolution MS^n data were acquired for the spiked chemical warfare agents, with IMS profile S/N ratios as high as 60:1.

Experimental

Samples and sample handling

Standard stock solutions of each chemical warfare agent were prepared in dichloromethane at 1 or 2 mg/mL. Individual chemical warfare agents (5 μg; either 2.5 or 5 μL) were spiked separately onto the glass surface of a 20 mL headspace sampling vial or onto media (Dacron swab, office furniture fabric, cardboard) contained within a 20 mL headspace sampling vial. The vial was capped following evaporation of the of solvent associated with the spike. Vials were heated to 40^{o}C (more volatile G-agents: sarin, soman, cyclohexyl sarin and tabun) or 80^{o}C (less volatile V-agent: VX) and sampled for 1 minute with a conditioned Supelco carbowax/divinyl benzene SPME fiber. The SPME fiber (housed within a Supelco SPME manual injector) was introduced directly into the ESI plume (acetonitrile/water, 10 μL/min) through a septum port in a plexiglass ESI housing (replacement) plate. This "in house" source modification facilitated the safe introduction and analysis of SPME fibers contaminated with chemical warfare agents.

Instrumental

DESI-MS data were acquired using a Synapt HDMS^{TM} tandem mass spectrometers (Waters, Manchester, UK) equipped with a Z-spray electrospray interface. The electrospray capillary was operated at 3 kV. Nitrogen desolvation gas (100 ^{o}C) was introduced into the interface (80 ^{o}C) at a flow rate of 300 L/h and nitrogen nebulizer gas was introduced at a flow rate of 50 L/h. MS^{n} data were acquired from m/z 40 to m/z 200 (or m/z 300) for the protonated molecular ion of each of the spiked chemical warfare agents. All MS^{n} data were acquired in the continuum mode with a resolution of 8000 (V-mode, 50% valley definition) with a scan rate of 0.5 s/scan.

The conditions used to meet instrument specifications were typically more energetic than required for smaller molecules such as chemical warfare agents and related compounds. A number of key parameters were investigated and reasonable setting(s) were determined for either MS^{n} or IMS-MS^{n} analysis of chemical warfare agents. Table 1 lists suggested settings and their impact.

Table 1: Key MS parameters investigated for chemical warfare agent (small molecule) analysis.

Synapt parameter	MS^n (without IMS)	IMS-MS^n (this study)	Reasoning
Sampling cone	≥ 15 V	15 V	The sampling cone was generally set to a low value to minimize product ion formation in the ESI source and facilitate selection of the $[M+H]^+$ ion. Settings below 15 V resulted in lower transmission.
Extraction cone	3 V	3 V	The extraction cone was reduced from the default setting (5 V) to minimize product ion formation in the ESI source and facilitate selection of the $[M+H]^+$ ion. Settings below 3 V resulted in lower transmission.
Trap collision energy	≥ 1.8 eV	3 eV	The minimum setting allowing good transmission and minimum fragmentation during MS (or MS/MS) analysis was 1.8 eV. A slightly higher energy, 3 eV, was required during IMS study. Higher settings resulted in enhanced product ion formation.
Trap gas flow	He or Ar: 1.4 mL/min	Ar: 4.5 mL/min	A valve was installed to select the trap gas. The use of helium results in much lower product ion formation, however resolution deceased by 25% over that with argon. Argon was used for IMS-MS^n study.
IMS gas flow		He: 35 mL/min	Helium replaced nitrogen as the IMS gas since the presence of nitrogen (even with the IMS off) resulted in significant product ion formation.
Transfer collision energy	≥ 0.2 eV	3 eV to 20 eV	The minimum setting allowing good transmission and minimum fragmentation during MS (or MS/MS) analysis was 0.2 eV. A slightly higher energy, 1 to 3 eV, was required during IMS study. Higher settings resulted in enhanced product ion formation.
Low Mass (LM) Resolution	5 or 12	12	The default setting was 4.7 which gave a window of about 4 Da. A setting of 5 was more appropriate for small molecules and gave a window of about 2 Da (with a 33% reduction in signal). A setting of 12 gave a window of 1 Da (with a 46% reduction in signal). A setting of 12 was used to reduce chemical interferences.

Results and Discussion

The first application of DESI-MS for chemical warfare agent analysis was reported by DRDC Suffield in 2005 at the ASMS [56]. Since that time the technique has been used at DRDC Suffield for the direct analysis of SPME fibers exposed to samples collected during scenario-based training in the field, and a variety of spiked office environment [33, 34] and consumer product [57] samples. A "dip and shoot" method was also developed for the direct analysis of fused silica glass or stainless steel capillaries. The capillaries were dipped into organic or aqueous samples containing chemical warfare agents, their hydrolysis products and related compounds and rapidly analysed by DESI-MS [58].

SPME forms a cornerstone in the analytical strategy developed by Canada for the identification of chemical warfare agents under realistic field sampling and/or analysis conditions. Samples, including swabs, liquids, soils and other materials taken in the field, would typically be contained in a septum-sealed vial, the headspace above which may be sampled with a SPME fiber, using a manual holder, without exposing the analyst to potentially harmful chemicals [47]. SPME headspace sampling can take as little as a few seconds for concentrated samples with higher volatility (i.e sarin). For lower volatility samples (i.e. VX), particularly at low concentration levels, heating and sampling times of five or more minutes may be required.

Analysis of SPME fibers would typically be performed by fast GC-MS and confirmed by DESI-MS and/or LC-ESI-MS [33, 34]. DESI-MS could be considered for primary analysis for a larger number of samples, as analysis times are typically minutes faster than even fast GC-MS. This technique can also be used for the detection and identification of both organophosphorus chemical warfare agents and their hydrolysis products without the need for derivatization steps [6]. The actual unmodified hydrolysis products may be confirmed by DESI-MS with significantly reduced sample handling and analysis time over fast GC-MS methods that require additional step(s) associated with derivatization.

Most DESI-MS analyses, including those performed with commercial interfaces, have been performed on an open stage that exposes the operator to the sample being introduced and the electrosprayed solvents. This was a concern when working with toxic chemicals, often at unknown concentrations, and a modification was made to the Z-spray ESI source of the Synapt HDMD^{TM} instrument to enable safe sample introduction. The aluminum side plate associated with the Z-spray source (lock mass probe side) was removed and a replica was machined using Plexiglass. A septum port was then installed to facilitate safe introduction of a SPME fiber held within a Supelco manual holder. Figure 1 illustrates the introduction of a SPME fiber into the ESI source for DESI-MS analysis. Positioning of the SPME fiber in the ESI plume was relatively easy and the exact position was not critical as the SPME fiber was relatively narrow and caused little disruption of the ESI plume.

Figure 1: DESI analysis of a SPME fiber in the Z-spray source of the Synapt HDMS (Inset: full view).

A high degree of flexibility has been incorporated into the Synapt instrument with its unique collision cell, allowing the operator to perform a variety of MS^n experiments. Product ion formation can take place in the ESI source, with higher sampling cone voltages resulting in increased product ion formation. The $[M+H]^+$ ion for a chemical warfare agent was most abundant at lower sampling cone voltages and a lower voltage (15 V) that maintained the $[M+H]^+$ ion was selected for all but a few additional confirmatory experiments. The quadrupole mass analyser can mass select with varying window widths and a m/z window of 1 (LM=12) was used to reduce chemical noise. The mass selected $[M+H]^+$ ion (or product ion formed in the ESI source) may undergo fragmentation in the trap collision region prior to the IMS cell. A generally

low setting that maintained significant $[M+H]^+$ intensity and also resulted in the formation of the principal product ion(s) was selected. The $[M+H]^+$ ion and one (or two) product ions exiting the trap collision region were then rapidly separated (9 msec) in the IMS cell on the basis of their ion mobility, resulting in the acquisition of a characteristic ion mobility profile. Finally the ion mobility separated ions were fragmented in the transfer collision region, generally at both a higher and lower energy setting, to facilitate the acquisition of MS^n data containing as many characteristic product ions as practical. With the higher transfer collision energy, fragmentation of the $[M+H]^+$ ion was often complete, leading to the formation of lower mass product ions that have not generally been acquired during most LC-ESI-MS analyses [6]. Significant fragmentation of the ion mobility separated principal product ion(s) also occurred with a higher transfer collision energy setting. DESI-IMS-MS^n analysis of a SPME fiber containing a chemical warfare agent enabled acquisition of an ion mobility profile for the compound and up to six MS^n full scanning, high resolution mass spectra, containing the $[M+H]^+$ ion for the compound and at least three other characteristic product ions that could be used to confirm the presence of the chemical warfare agent. Acquisition of IMS and MS^n data in this manner has been referred to as time-aligned parallel (TAP) fragmentation. Errors associated with mass measurement were generally less than 2 mDa, typical of those reported during prior experiments [33] and by Williams and Scrivens [26].

Figure 2 illustrates the data generated during the DESI-IMS-MS^n analysis of a SPME fiber exposed to the headspace above 5 µg of cyclohexyl sarin (1 min at 40°C). DESI- MS^n data were acquired for approximately two minutes following insertion of the SPME fiber into the ESI plume. Detection was immediate and sufficient signal was collected within a few seconds to confirm the presence of cyclohexyl sarin. The $[M+H]^+$ at m/z 181 was selected with the quadrupole mass analyser and allowed to fragment under relatively low energy conditions (3 eV) in the trap collision region. A significant product ion at m/z 99, due to loss of C_6H_{10}, was observed. Both the m/z 181 and m/z 99 ions were resolved in the helium-filled IMS cell with bin numbers of 60 and 86, respectively (200 bins = 9 msec). The ion mobility separated ions were passed into the transfer collision region, set at 3eV, where only fragmentation of the m/z 181 ion was significant. Higher energy would be required to fragment the m/z 99 ion.

Lower energy settings in the collision regions favoured preservation of the $[M+H]^+$ ion for the chemical warfare agents and these settings were used to compare the IMS profiles of the five organophosphous chemical warfare agents following DESI-IMS-MS^n analysis. Figure 3 illustrates the unique profiles acquired for each of the compounds following analysis of SPME fibers exposed to the headspace above each chemical warfare agent (5 µg in a 20 mL glass headspace sampling vial). The ion mobility profiles were all different and could be used to aid in compound confirmation. Bins numbers were unique for each compound and the m/z ratio of the ion responsible has been indicated on each ion mobility profile. The m/z 99 ion, due to loss of the alkene associated with the alkoxy group for each of the methylphosphonofluoridates (GB, GF and GD), was consistently observed at bin number 60. The $[M+H]^+$ ion was observed in bin number 75 for GB, bin number 81 for GA, bin number 85 for GF, bin number 86 for GD and bin number 105 for VX. A product ion due to loss of ethylene (m/z 135) was observed for GA at bin number 72. GD exhibited an additional product ion at m/z 85 (bin number 68) due to $[C_6H_{13}]^+$ and VX was characterized by the presence of a product ion at m/z 128 (bin number 76) due to $[C_2H_4N(iPr)_2]^+$. Reproducibility over a two month period, including a scheduled shut-down, was generally good with bin numbers being the same. Differences, when observed, did not exceed ± 1 bin number over this time period.

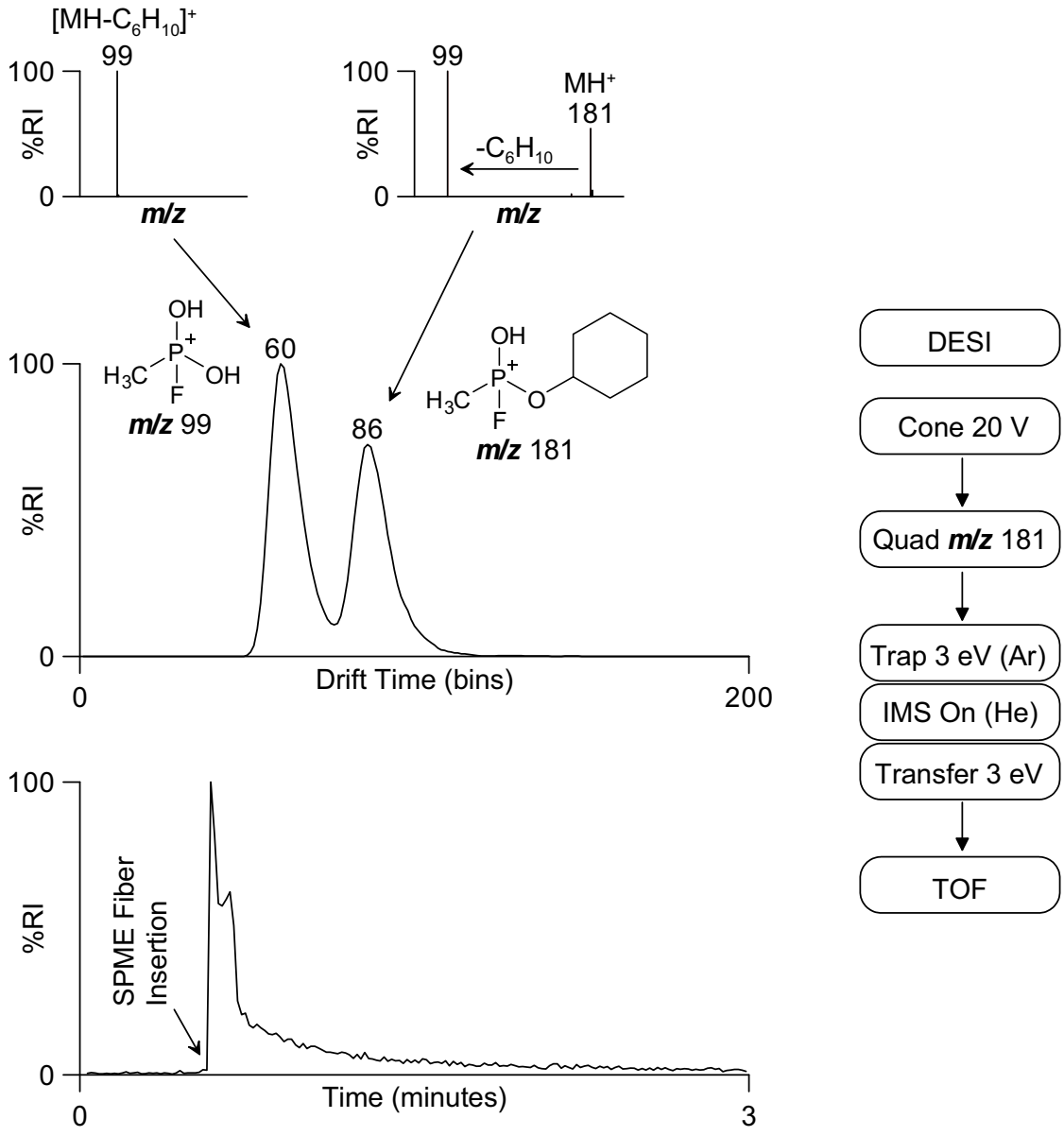

Figure 2: DESI-IMS-MS^n analysis of a SPME fiber used to sample (1 min at 40^{o}C) the headspace above 5 µg of cyclohexyl sarin in a glass headspace sampling vial. Lower: Total-ion-current chromatogram for m/z 181, [M+H]$^+$ ion for cyclohexyl sarin, selected by the quadrupole analyser. Upper: Ion mobility profile (200 bins = 9 msec.) for cyclohexyl sarin, and the acquired MS/MS spectrum for each of the separated ions (m/z 181 and m/z 99) with a transfer energy setting of 3 eV.(% RI - % Relative Intensity)

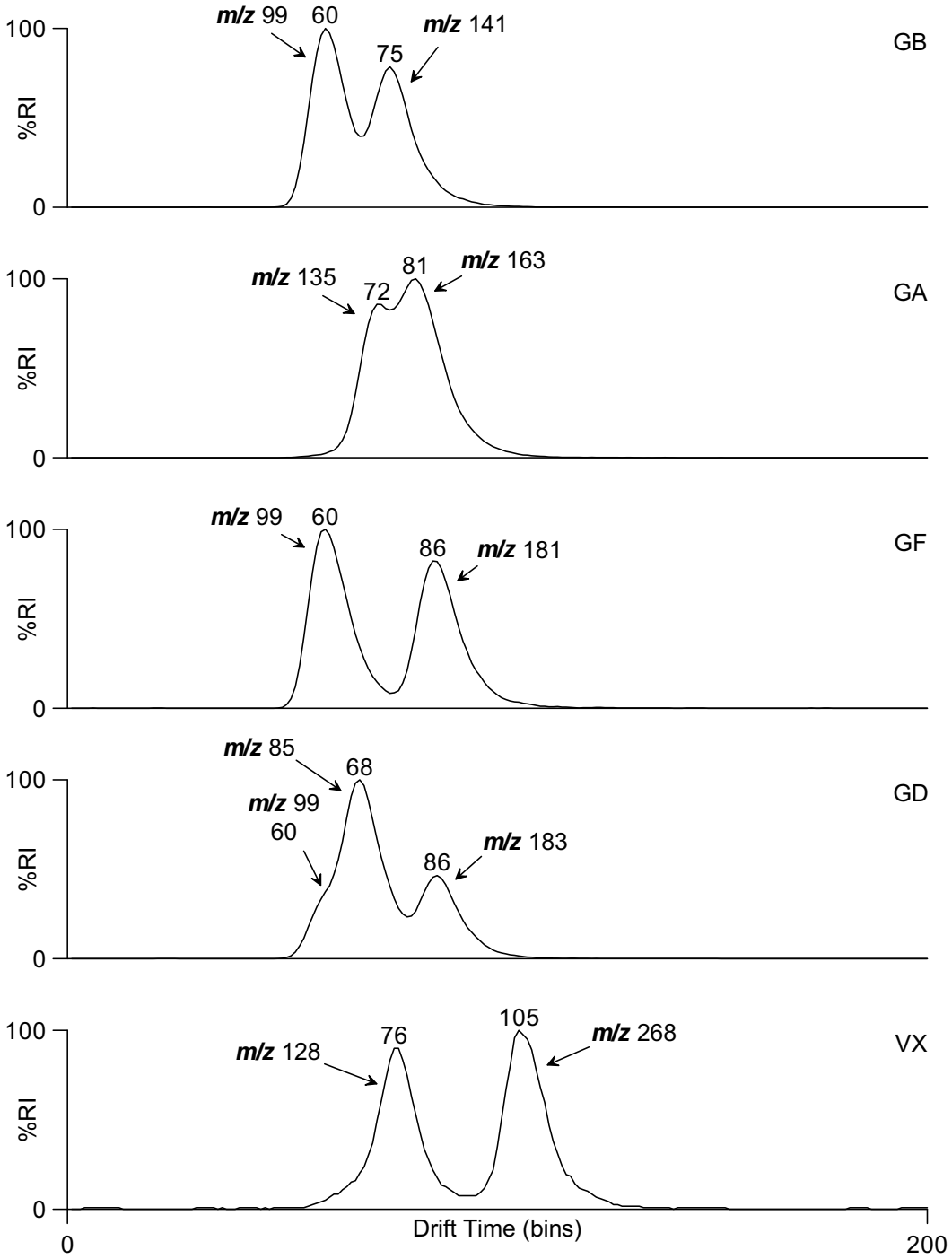

Figure 3: Ion mobility profiles (200 bins = 9 msec.) obtained for sarin (GB), tabun (GA), cyclohexyl sarin (GF), soman (GD) and VX following DESI-IMS- MS^n analysis of SPME fibers exposed to the headspace above 5 µg each compound. The $[M+H]^+$ ion selected by the quadrupole analyser and one or more product ions, generated in the trap region of the collision cell, were separated by IMS (m/z values indicated).

Figures 4 to 8 illustrate the MS^n data acquired and the fragmentation pathways associated with the analysis of each chemical warfare agent. Between four and eight characteristic ions, including the $[M+H]^+$ ion, were observed in the MS^n data acquired for each compound during DESI-IMS-MS^n analysis.

Figure 4 illustrates MS^n data obtained during DESI-IMS-MS^n of sarin, headspace sampled on a SPME fiber for 1 min at 40°C. Data were acquired with a sampling cone voltage of 15 V and a trap collision energy setting of 3 eV. Every 0.5 s the transfer collision energy was alternated between 3 eV and 20 eV to enable acquisition of lower mass product ions. The $[M+H]^+$ ion at m/z 141, was resolved by ion mobility from the m/z 99 ion formed following loss of C_3H_6 in the trap collision region. Following ion mobility separation, the m/z 141 ion fragmented to produce only the m/z 99 ion with a transfer collision energy setting of 3 eV. A higher transfer collision energy setting of 20 eV resulted in formation of a $C_3H_7^+$ ion and product ions at m/z 81 and m/z 79 due to loss of H_2O and HF, respectively, from the m/z 99 ion. The higher setting also resulted in enhanced fragmentation of the m/z 99 ion resolved following IMS. Significant product ions were again observed at m/z 81 and m/z 79, as well as an ion at m/z 47 due to $[PO]^+$ that could be formed from either m/z 81 or m/z 79. This was confirmed by using a higher sampling cone voltage, 60V, that resulted in formation of both the m/z 81 and m/z 79 ions in the ESI source. Each ion was then selected (individual analyses) with the quadrupole mass analyser (LM=12) and fragmented using a transfer collision energy setting of 10 eV. The m/z 47 ion was the only significant product ion formed for both m/z 81 and m/z 79. The elemental composition of all six sarin ions observed in the MS^n data was confirmed by comparing acquired high resolution data with calculated values.

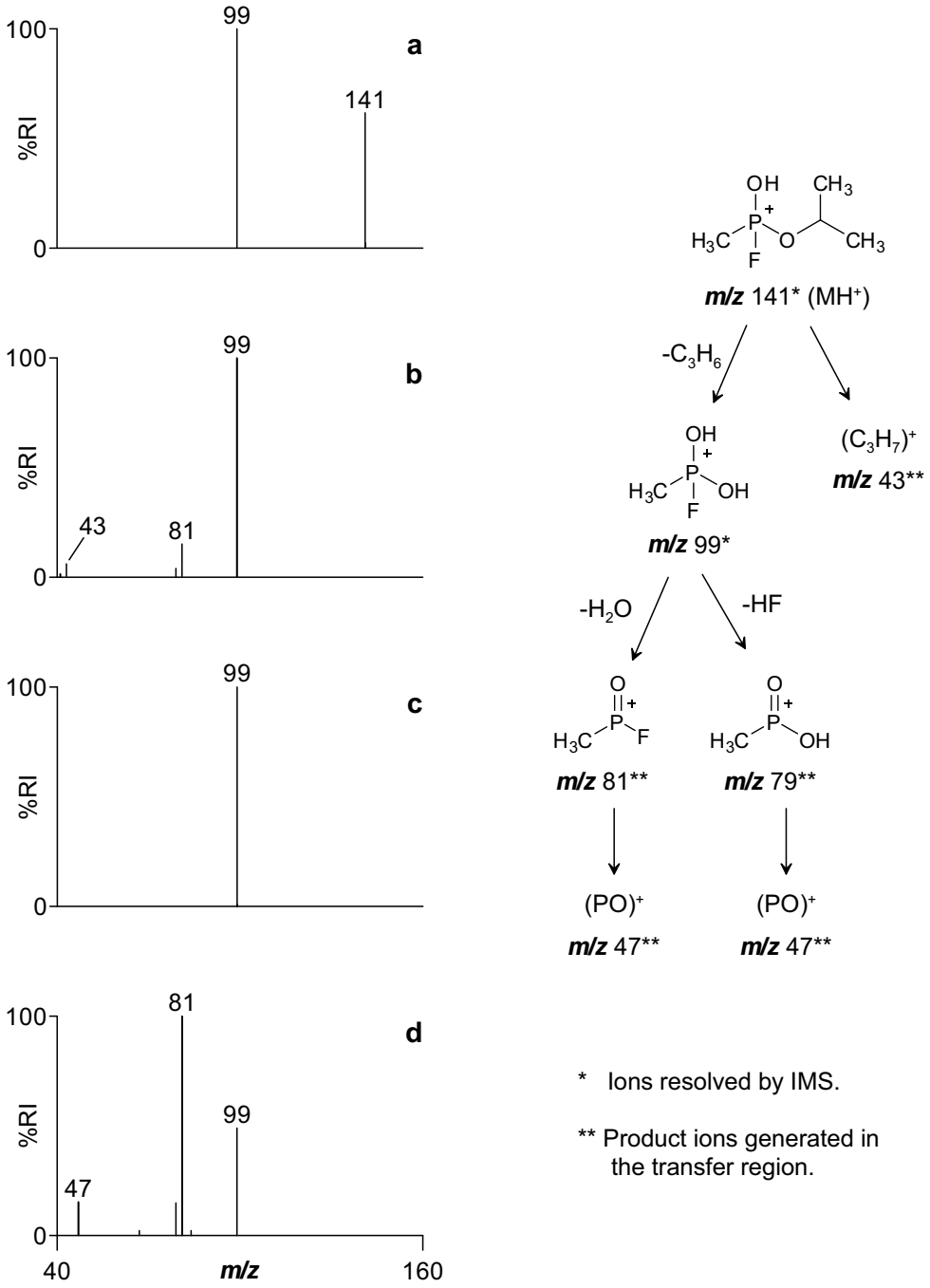

Figure 4: DESI-IMS MS^n analysis of a SPME fiber used to sample (1 min at 40°C) the headspace above 5 µg of sarin in a glass headspace sampling vial. The $[M+H]^+$ ion for sarin (m/z 141) was selected by the quadrupole analyser and the m/z 99 ion was generated with a trap collision energy of 3 eV. The m/z 141 and m/z 99 were separated by IMS. MS^2 spectrum of a) m/z 141 and MS^3 spectrum of c) m/z 99 with a lower transfer collision energy, 3 eV. MS^2 spectrum of b) m/z 141 and MS^3 spectrum of d) m/z 99 with a higher transfer collision energy, 20 eV.

Similar MS^n data were acquired for the other four chemical warfare agents. Figure 5 illustrates the MS^n data acquired for cyclohexyl sarin. Both the $[M+H]^+$ ion, at m/z 181, and the product ion due to loss of C_6H_{10}, at m/z 99, for cyclohexyl sarin (GF) were resolved by IMS. Higher transfer collision energy resulted in the formation of ions at m/z 83, due to $[C_6H_{11}]^+$, and m/z 55, due to $[C_4H_7]^+$, from the m/z 181 ion. The same product ions observed for the m/z 99 ion for sarin were observed for cyclohexyl sarin with the higher transfer collision energy setting.

Figure 6 illustrates the MS^n data acquired for soman. Three ions, at m/z 183, m/z 99 and m/z 85, were resolved by IMS for soman (GD). The $[M+H]^+$ ion, at m/z 183, exhibited the common methylphosphonfluoridate product ion at m/z 99, and product ions at m/z 85, m/z 57 and m/z 43, due to $[C_6H_{13}]^+$, $[C_4H_9]^+$ and $[C_3H_7]^+$, respectively, with the lower mass product ions being more significant at the higher transfer collision energy, 20 eV. The same product ions observed for the m/z 99 ion for sarin and cyclohexyl sarin were observed for soman with the higher transfer collision energy setting. The m/z 85 ion, due $[C_6H_{13}]^+$, fragmented to produce product ions at m/z 43 and m/z 41, due to $[C_3H_7]^+$ and $[C_3H_5]^+$, respectively.

Figure 7 illustrates the MS^n data acquired for tabun. Both the $[M+H]^+$ ion at m/z 163 and the product ion due to loss of C_2H_4, at m/z 135 for tabun were resolved by IMS. Product ions due to loss of H_2O and HCN from the m/z 135 ion were also observed at m/z 117 and m/z 108, respectively.

Figure 8 illustrates the MS^n data acquired for VX. VX, a less volatile chemical warfare agent was headspace sampled for 1 minute at 80°C, as opposed to 40°C for the other more volatile compounds. A higher energy setting in the trap collision region (15 eV) was required to fragment the $[M+H]^+$ ion at m/z 268. The m/z 268 ion and the m/z 128 ion, due to loss of the acid, $(O)P(OC_2H_5)(SH)(CH_3)$, were resolved by IMS and fragmented effectively with a relatively high transfer collision setting of 18 eV. Product ions at m/z 86 and m/z 44, due to sequential loss of C_3H_6, were observed for the ion at m/z 128. An ion at m/z 167, due to loss of $HN(iPr)_2$ from the $[M+H]^+$ ion, as well as ions at m/z 128 and m/z 86 were observed for the m/z 268 ion.

The acquired time-aligned parallel (TAP) fragmentation data acquired during DESI-IMS-MS^n analysis of each chemical warfare agents, sarin, tabun, cyclohexyl sarin, soman and VX has been summarized with Table 2. It should also be noted that the bin windows associated with the IMS separation consistently decreased by about 2 bin numbers with higher transfer collision energies.

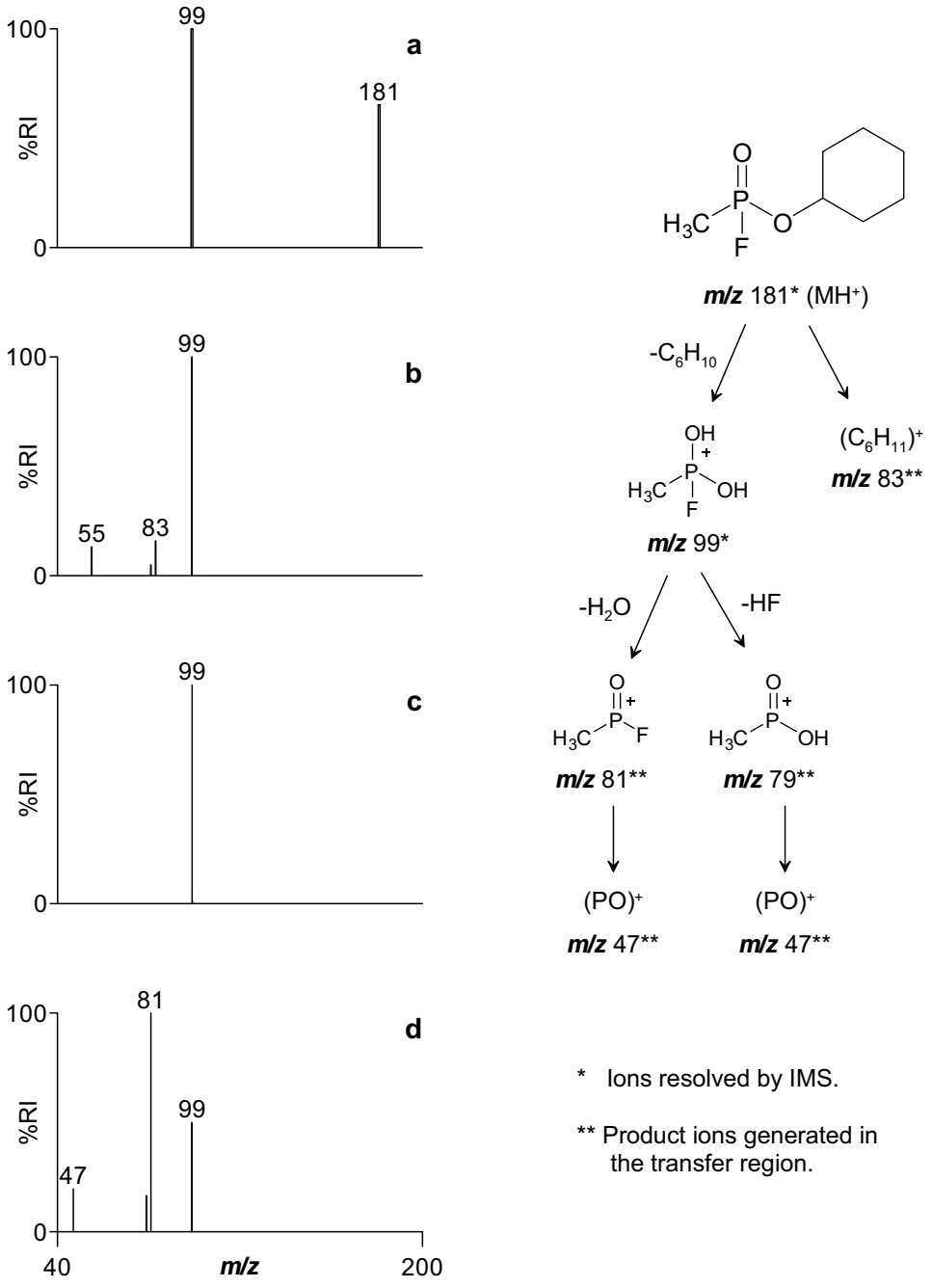

Figure 5: DESI-IMS-MS^n analysis of a SPME fiber used to sample (1 min at 40^{o}C) the headspace above 5 µg of cyclohexyl sarin in a glass headspace sampling vial. The [M+H]$^+$ ion for cyclohexyl sarin (m/z 181) was selected by the quadrupole analyser and the m/z 99 ion was generated with a trap collision energy of 3 eV. The m/z 181 and m/z 99 were separated by IMS. MS^2 spectrum of a) m/z 181 and MS^3 spectrum of c) m/z 99 with a lower transfer collision energy, 3 eV. MS^2 spectrum of b) m/z 181 and MS^3 spectrum of d) m/z 99 with a higher transfer collision energy, 20 eV.

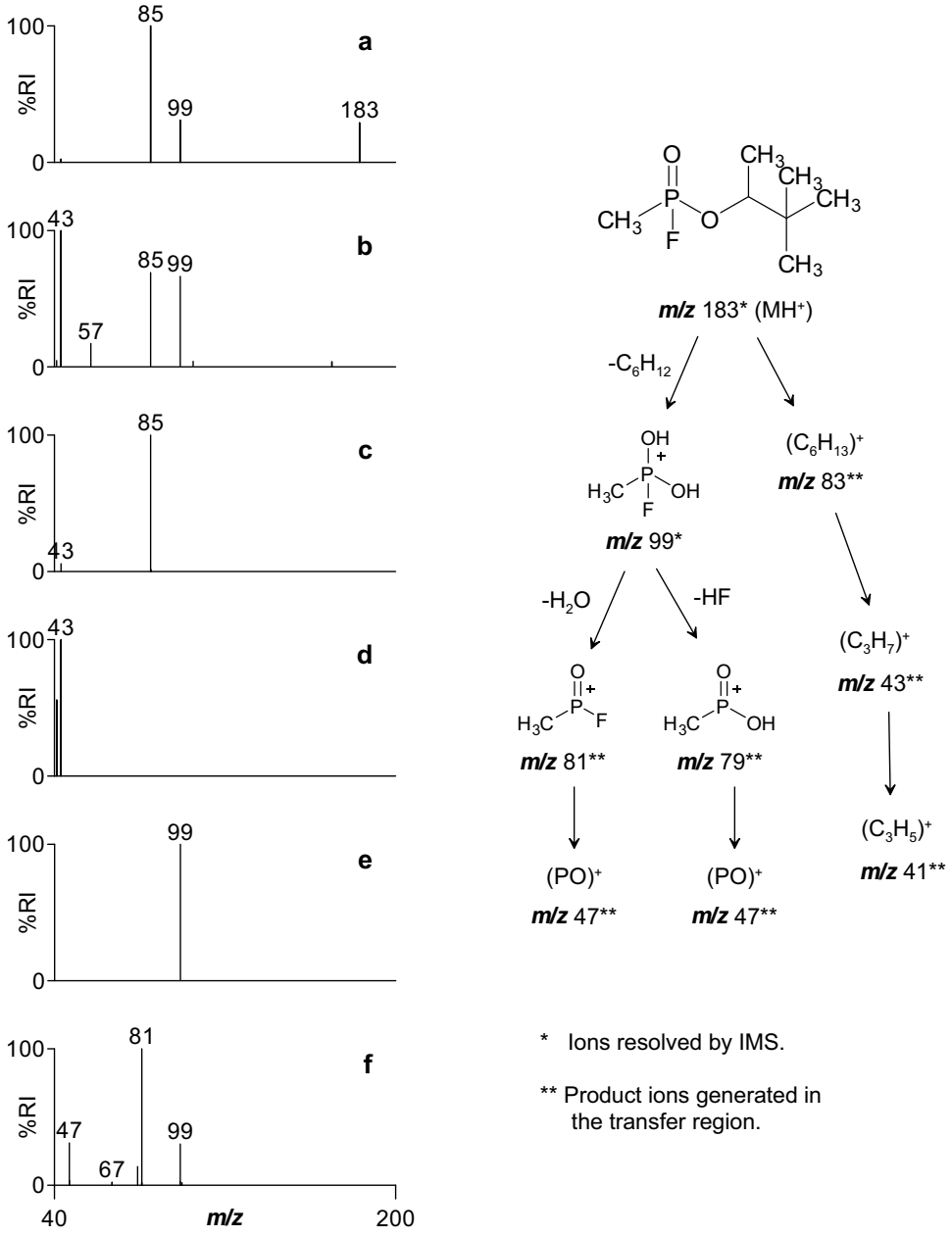

Figure 6: DESI-IMS-MS^n analysis of a SPME fiber used to sample (1 min at 40^{o}C) the headspace above 5 µg of soman in a glass headspace sampling vial. The [M+H]$^+$ ion for soman (m/z 183) was selected by the quadrupole analyser and the m/z 99 and m/z 85 ions were generated with a trap collision energy of 3 eV. The m/z 183, m/z 99 and m/z 85 ions were separated by IMS. MS^2 spectrum of a) m/z 183 and MS^3 spectrum of c) m/z 99 and e) m/z 85 with a lower transfer collision energy, 3 eV. MS^2 spectrum of b) m/z 183 and MS^3 spectrum of d) m/z 99 and f) m/z 85 with a higher transfer collision energy, 20 eV.

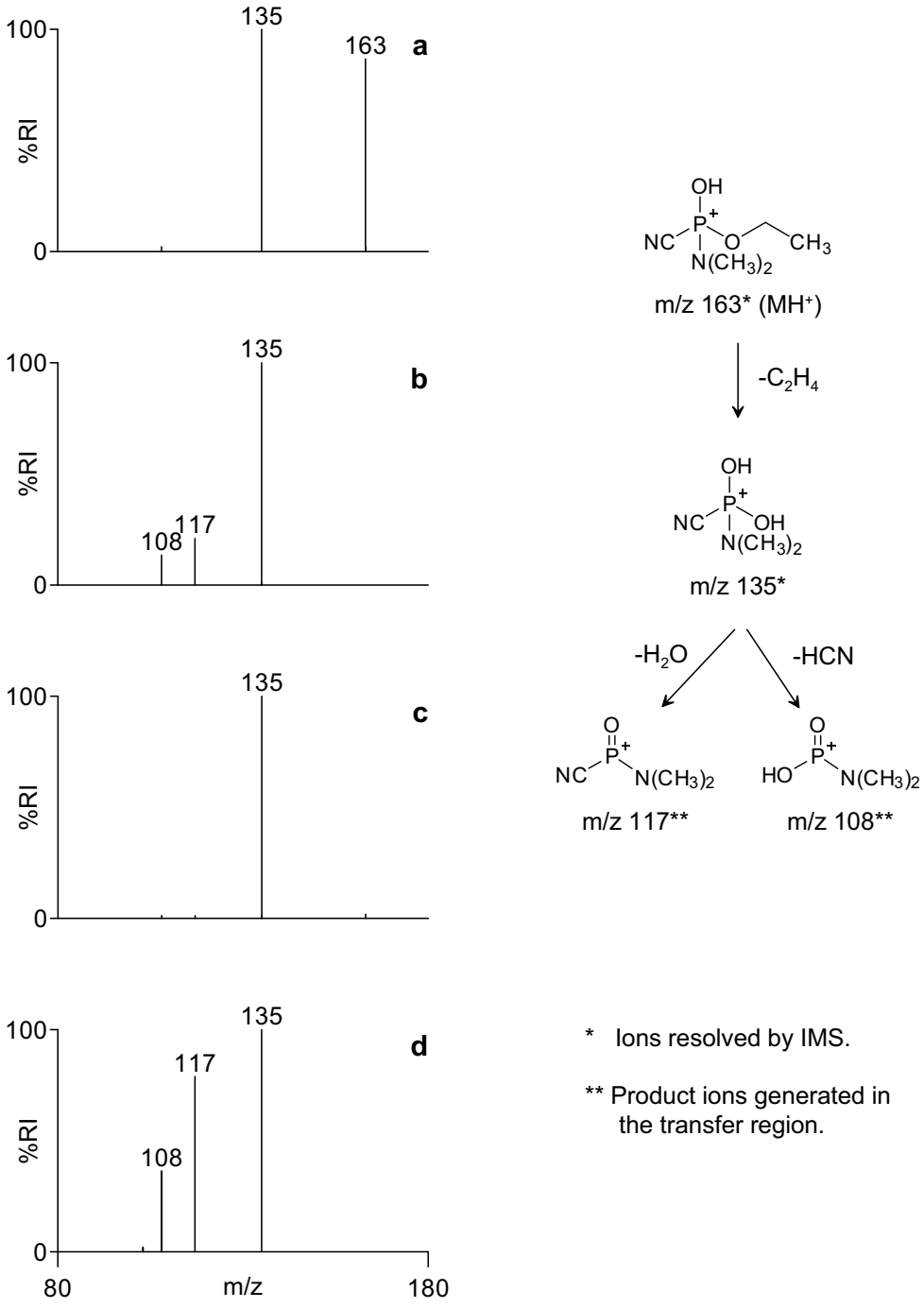

Figure 7: DESI-IMS-MS^n analysis of a SPME fiber used to sample (1 min at 40^{o}C) the headspace above 5 µg of tabun in a glass headspace sampling vial. The [M+H]$^+$ ion for tabun (m/z 163) was selected by the quadrupole analyser and the m/z 135 ion was generated with a trap collision energy of 7 eV. The m/z 163 and m/z 135 were separated by IMS. MS^2 spectrum of a) m/z 163 and MS^3 spectrum of c) m/z 135 with a lower transfer collision energy, 7 eV. MS^2 spectrum of b) m/z 163 and MS^3 spectrum of d) m/z 135 with a higher transfer collision energy, 15 eV.

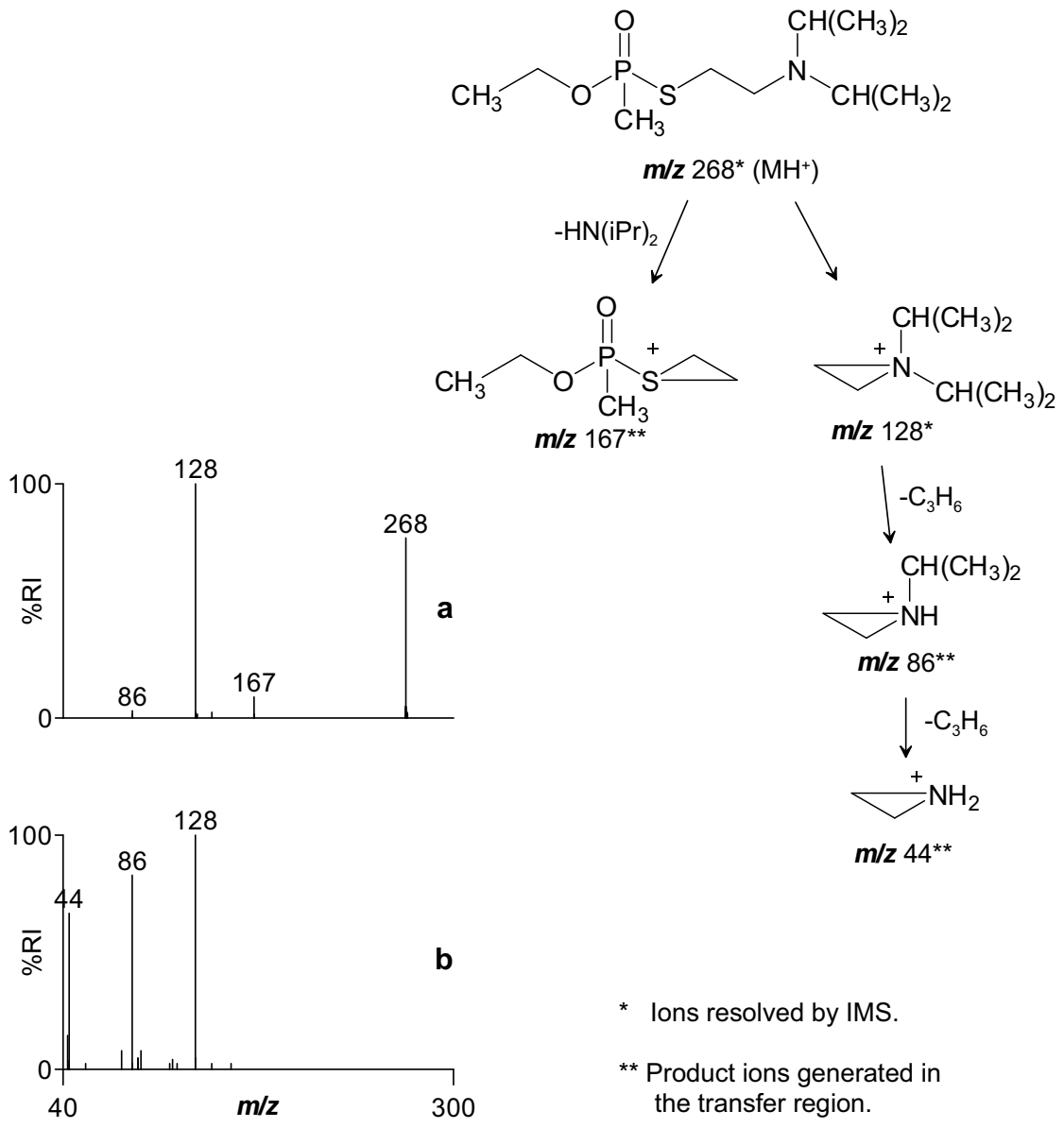

Figure 8: DESI-IMS-MS^n analysis of a SPME fiber used to sample (1 min at 80^{o}C) the headspace above 5 µg of VX in a glass headspace sampling vial. The [M+H]$^+$ ion for VX (m/z 268) was selected by the quadrupole analyser and the m/z 128 ion was generated with a trap collision energy of 15 eV. The m/z 268 and m/z 128 were separated by IMS. MS^2 spectrum of a) m/z 268 and MS^3 spectrum of b) m/z 128 with a transfer collision energy of 18 eV.

Table 2: Time-aligned parallel (TAP) fragmentation (MS^n) data acquired during DESI-IMS-MS^n analysis of each chemical warfare agent. Sarin (GB), tabun (GA), cyclohexyl sarin (GF) and soman (GD) were each headspace sampled at 40°C for 1 min from a headspace sampling vial. VX was sampled similarly at 80°C for 1 min.

Agent	Trap/Transfer Collision energies (eV)[a]	Precusor ion (Selected by Quadrupole) m/z	Ion Mobility Bin Number[b] (m/z value of ion separated by IMS)	TAP Fragmentation (MS^n) Data Acquired Following IMS Separation m/z(%Relative Intensity)
GB	3/3	141 $[M+H]^+$	60 (m/z 99) 75 (m/z 141)	MS^3 99(100) MS^2 141(62), 99(100)
	3/20	141 $[M+H]^+$	58 (m/z 99) 73 (m/z 141)	MS^3 99(51), 81(100), 79(16), 47(16) MS^2 99(100), 81(15), 79(4), 43(6)
GA	7/7	163 $[M+H]^+$	72 (m/z 135) 81 (m/z 163)	MS^3 135(100) MS^2 163(86), 135(100)
	7/15	163 $[M+H]^+$	71 (m/z 135) 80 (m/z 163)	MS^3 135(100), 117(78), 108(37) MS^2 135(100), 117(22), 108(13)
GF	3/3	181 $[M+H]^+$	60 (m/z 99) 86 (m/z 181)	MS^3 99(100) MS^2 181(66), 99(100)
	3/20	181 $[M+H]^+$	58 (m/z 99) 84 (m/z 181)	MS^3 99(50), 81(100), 79(17), 47(21) MS^2 99(100), 83(17), 81(6), 79(1), 55(13)
GD	3/3	183 $[M+H]^+$	60 (m/z 99) 68 (m/z 85) 86 (m/z 183)	MS^3 99(100) MS^3 85(100), 43(5) MS^2 183(30), 99(33), 85(100), 43(2)
	3/20	183 $[M+H]^+$	58 (m/z 99) 65 (m/z 85) 84 (m/z 183)	MS^3 99(28), 81(100), 79(15), 47(30) MS^3 43(100), 41(56) MS^2 99(66), 85(72), 57(20), 43(100)
VX	15/18	268 $[M+H]^+$	76 (m/z 128) 105 (m/z 268)	MS^3 128(100), 86(85), 44(66) MS^2 268(77), 167(10), 128(100), 86(4)

[a] Sampling cone of 15 V used for GB, GA, GF and GD. A higher sampling cone, 45 V, was used for VX to minimize chemical noise.

[b] 200 bins = 9 msec.

A screening procedure based on this investigation, using the lower transfer collision energy setting for each chemical warfare agent (refer to Table 2), was developed for the four G-agents (GA, GF, GB and GD). Unknowns submitted for SPME headspace sampling and DESI-IMS-MS^n analysis may be analysed for all four chemical warfare agents during a single analysis as opposed to four individual analyses. The sampling cone was maintained at 15 V, the quadrupole mass analyser was set to the m/z value of the $[M+H]^+$ ion for each compound during acquisition and the trap and transfer collision energies were 7 eV for GA and 3 eV for GF, GB and GD. Each of the four scan functions (0.5 s/scan) was acquired in an alternating manner during the analysis.

Media (Dacron swab, cardboard and office furniture fabric) whichmight be collected in an office environment during a forensic investigation [34] were spiked with 5 µg of chemical warfare agent, sampled for 1 min at 40°C with a SPME fiber, and analysed for the presence of all four chemical warfare agents within 1 min by DESI-IMS-MS^n. Spiking levels were typical of those employed by the Organization for the Prohibition of Chemical Weapons during proficiency testing, in the 10 µg/g or higher range.

Figure 9 illustrates the IMS profiles generated during screening of a Dacron swab blank (each swab weighs approximately 0.5 g) and a Dacron swab spiked with 5 µg of sarin. The IMS profiles collected during DESI-IMS-MS^n analysis of the blank swab were relatively free of chemical interferences, while the spiked swab exhibited a distinct ion mobility profile characteristic of sarin (bin numbers 60 and 75 observed as per results in Table 2). Signal to noise (S/N) in the ion mobility profile exceeded 60:1 for the sarin. The MS^n spectra (inset on Figure 9) were typical of those acquired during prior analysis of sarin spiked onto the glass surface of a headspace sampling vial (Figure 4a and 4c).

Similar blank and spiked sample results were obtained with the office furniture fabric (Figure 10) and cardboard (Figure 11) spiked with sarin. Signal to noise (S/N) in the ion mobility profile exceeded 60:1 for the sarin spiked onto the cardboard and exceeded 30:1 for sarin spiked onto the office furniture fabric. The sensitivities observed for the other chemical warfare agents spiked onto the media were lower, likely due to the fact that the agents have lower volatility than sarin. However, in all cases the spiked chemical warfare agent was readily identified by the comparing the acquired ion mobility profile and MS^n data with that acquired for standards (Table 2). Figures 12 and 13 illustrate the ion mobility profiles obtained during screening of a Dacron swab spiked with tabun (S/N > 20:1) and a Dacron swab spiked with cyclohexyl sarin (S/N > 10:1). Similar S/N ratios were also obtained during analysis of cardboard spiked with soman (S/N > 7:1). All the spiked chemical warfare agents were readily identified on the basis of the acquired MS^n spectra and IMS bin numbers were all within ± 1 of those reported in Table 2.

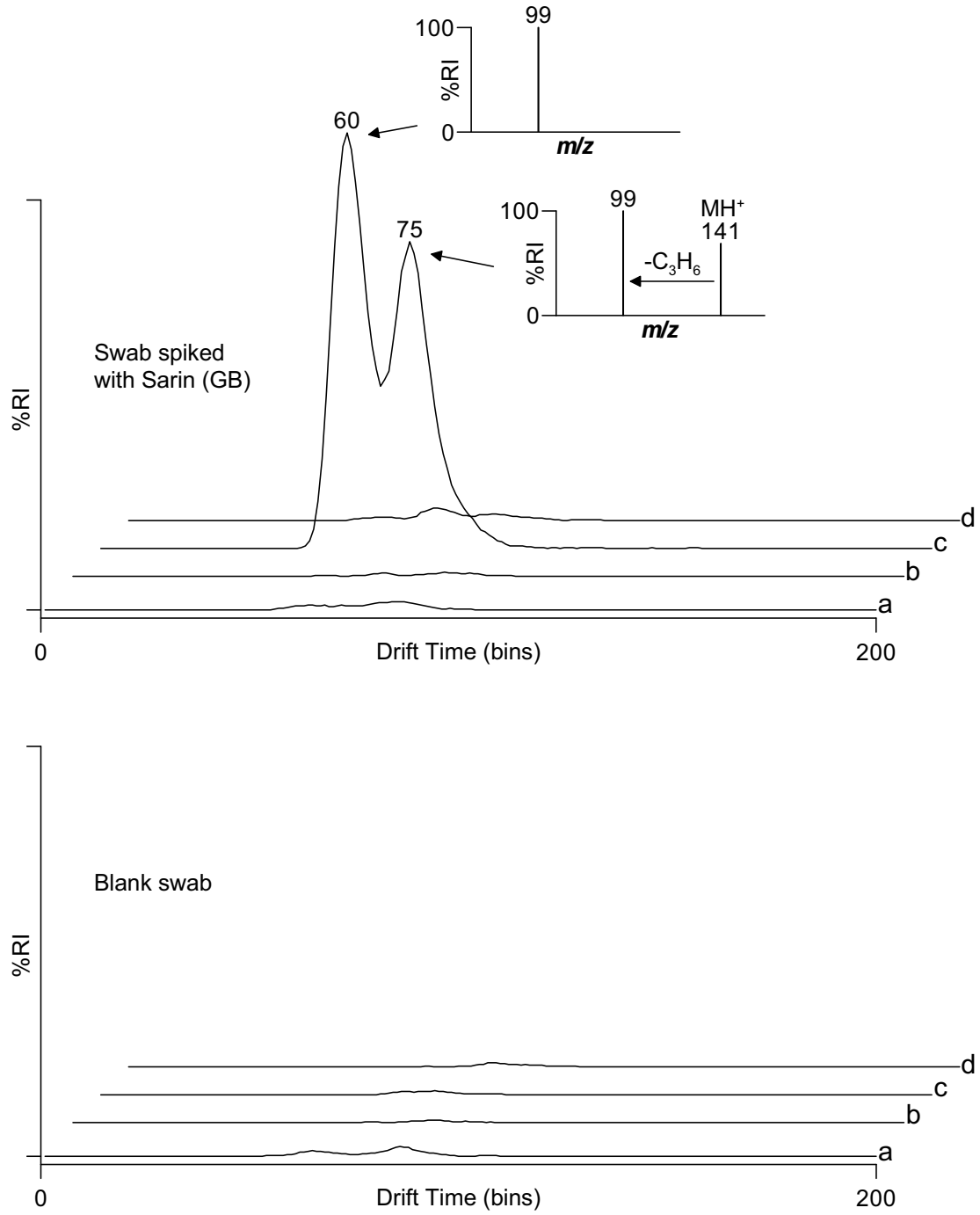

Figure 9: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above a blank Dacron swab and a Dacron swab spiked with 5 µg of sarin. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analysis with a lower transfer collision energies setting to insure preservation of the [M+H]$^+$ ion (if present). (Inset: acquired data for sarin).

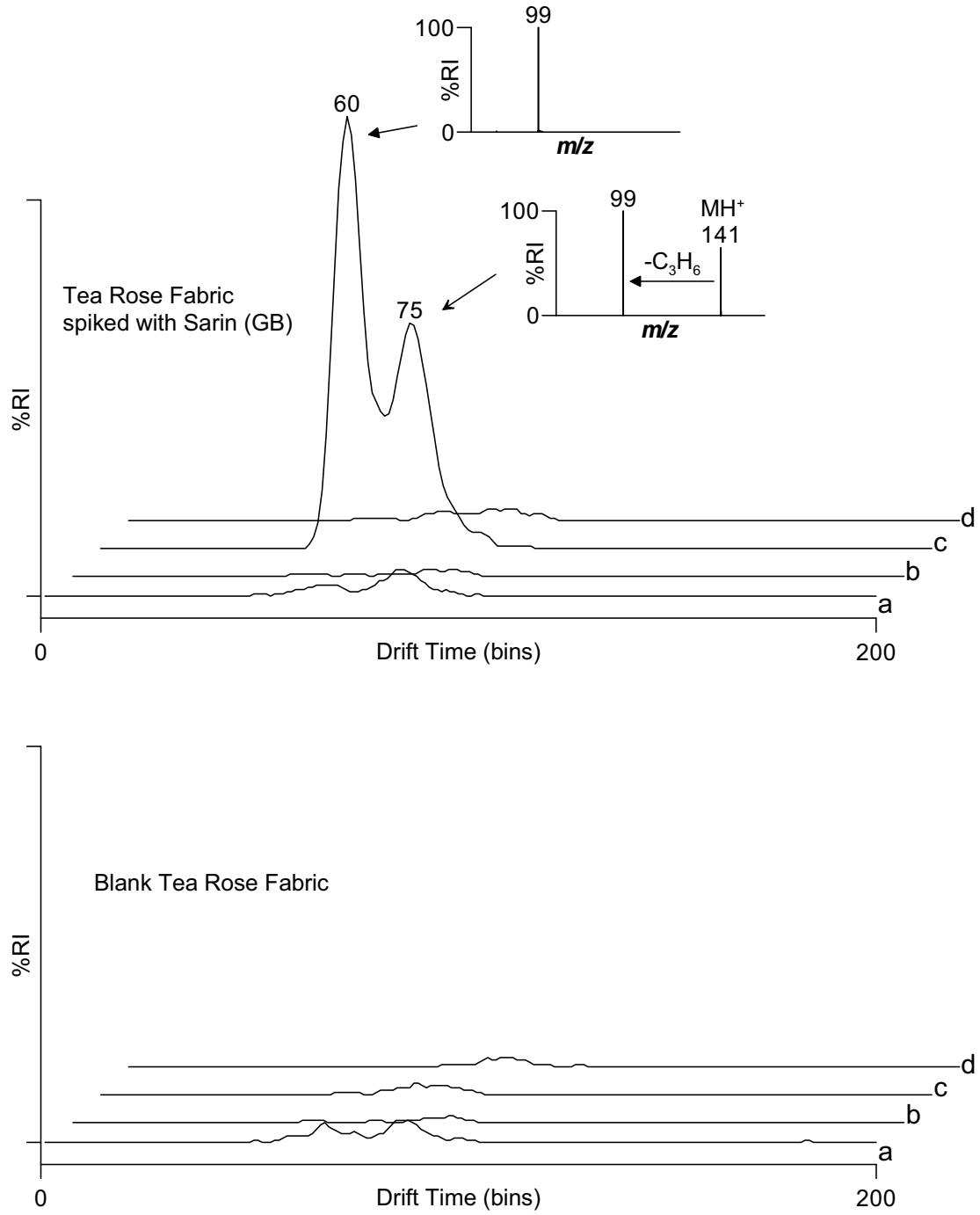

Figure 10: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above blank office furniture fabric and office furniture fabric spiked with 5 µg of sarin. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analysis with a lower transfer collision energies setting to insure preservation of the [M+H]$^+$ ion (if present). (Inset: acquired data for sarin).

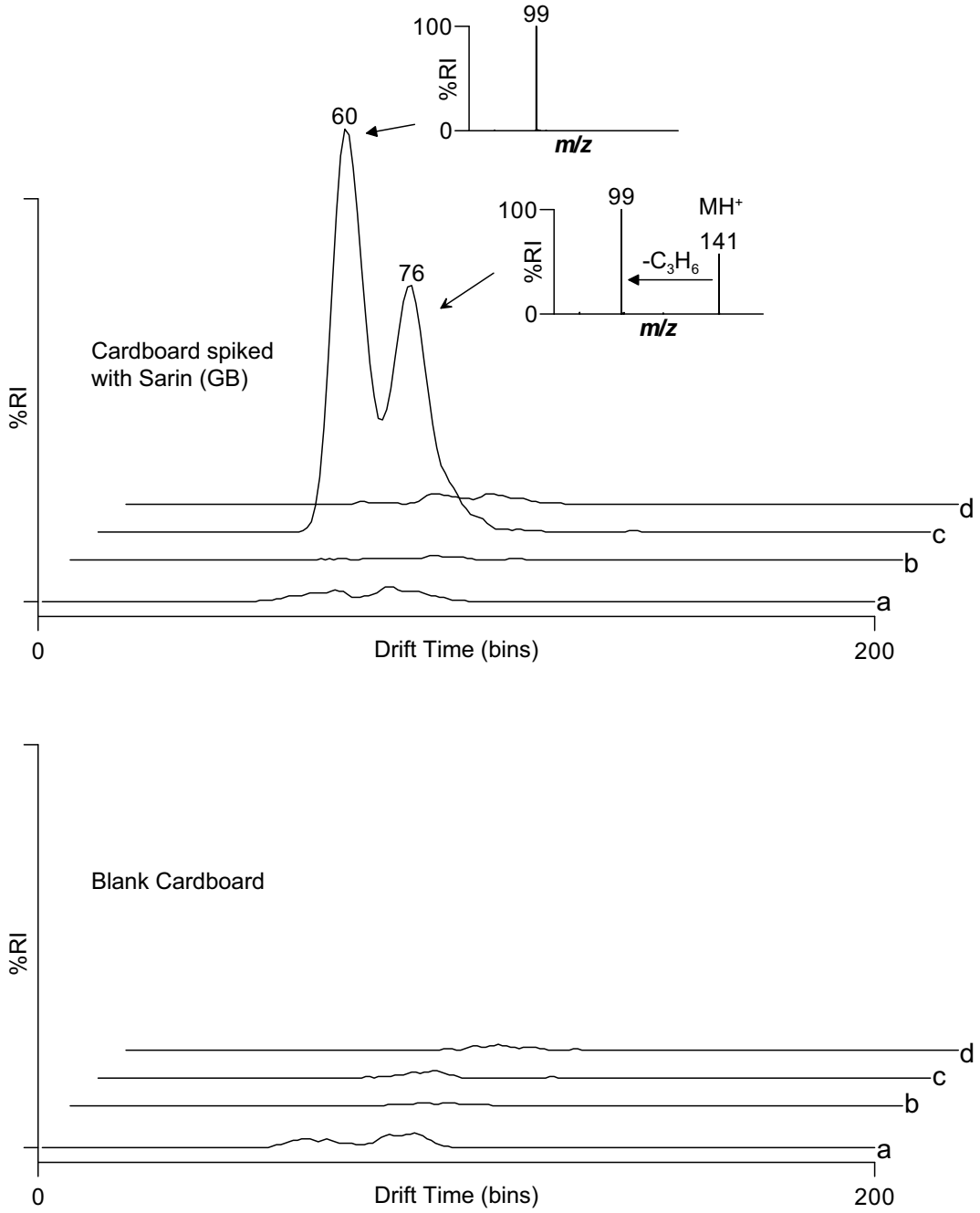

Figure 11: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above blank cardboard and cardboard spiked with 5 µg of sarin. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analysis with a lower transfer collision energies setting to insure preservation of the $[M+H]^+$ ion (if present). (Inset: acquired data for sarin).

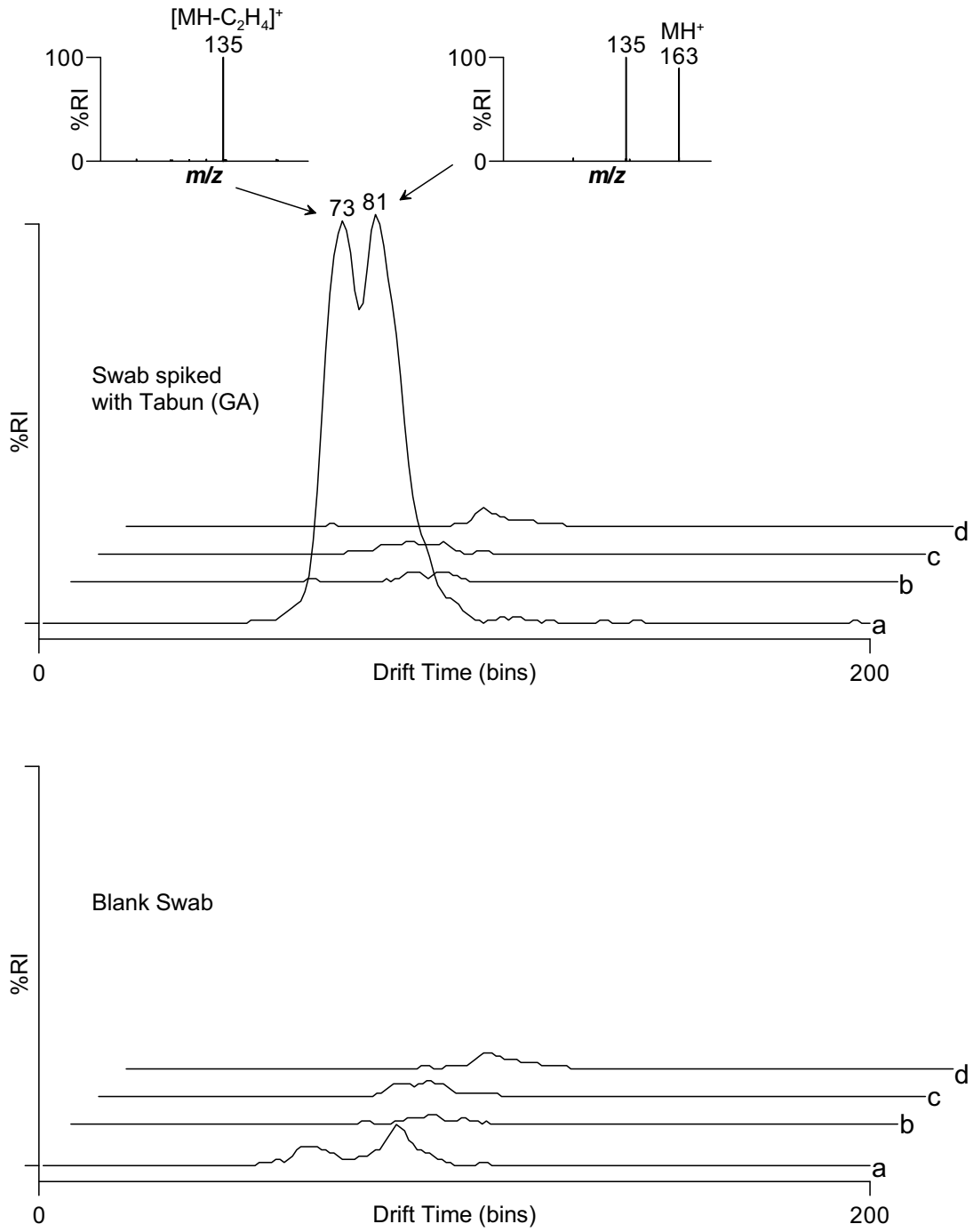

Figure 12: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above a blank Dacron swab and a Dacron swab spiked with 5 µg of tabun. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analsis with a lower transfer collision energies setting to insure preservation of the [M+H]$^+$ ion (if present). (Inset: acquired data for tabun).

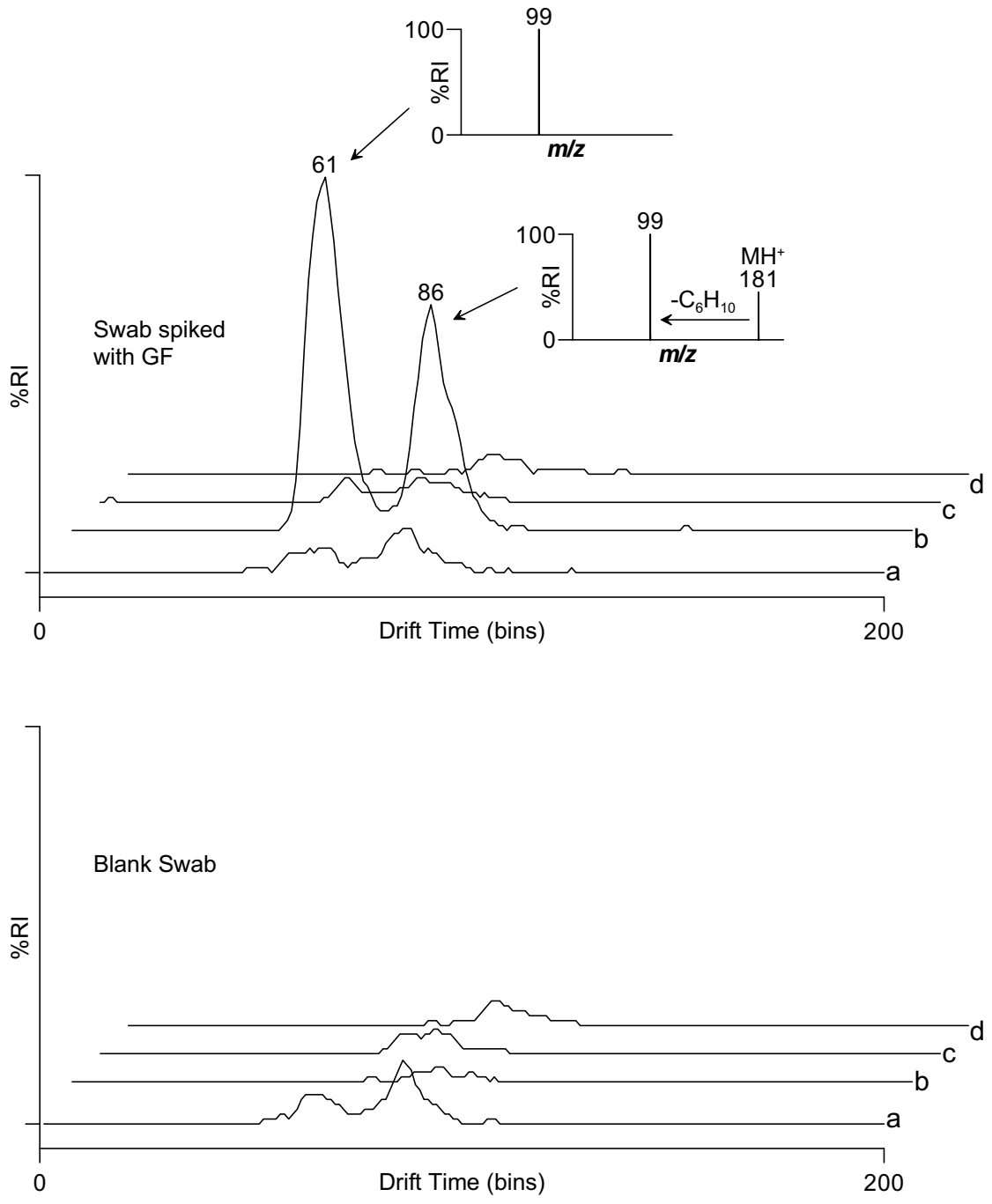

Figure 13: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above a blank Dacron swab and a Dacron swab spiked with 5 µg of cyclohexyl sarin. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analysis with a lower transfer collision energies setting to insure preservation of the $[M+H]^+$ ion (if present). (Inset: acquired data for cyclohexyl sarin).

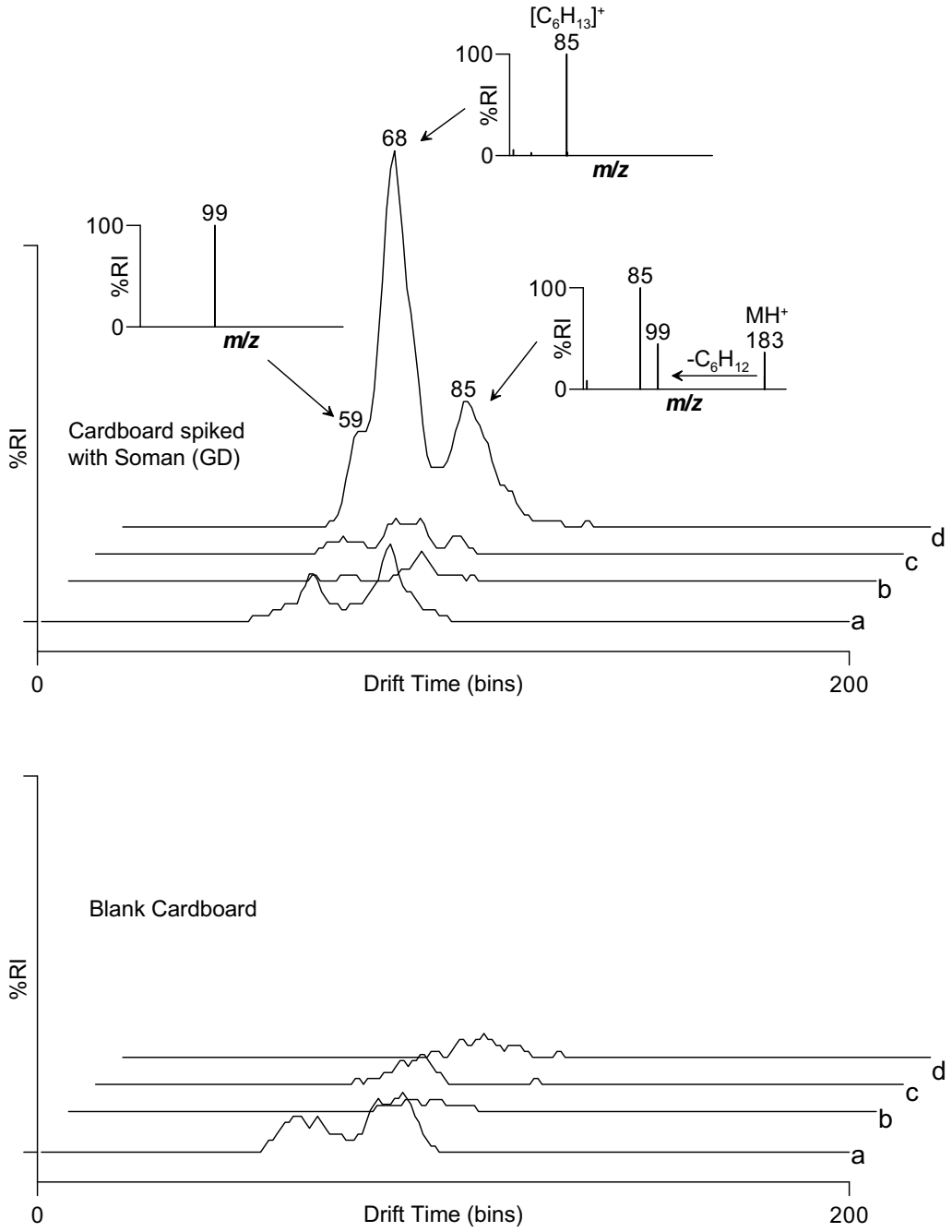

Figure 14: Ion mobility profiles obtained using the developed G-agent screening procedure following DESI-IMS-MS^n analysis of SPME fibers exposed to the headspace above blank cardboard and cardboard spiked with 5 µg of soman. The ion mobility profiles for the four G-agents a) tabun, b) cyclohexyl sarin, c) sarin and d) soman were acquired during each analysis ith a lower transfer collision energies setting to insure preservation of the $[M+H]^+$ ion (if present). (Inset: acquired data for soman).

Conclusions

Five organophosphorous chemical warfare agents, O-isopropyl methylphosphonofluoridate (sarin, GB), O-pinacolyl methylphosphonofluoridate (soman, GD), O-ethyl N,N-dimethylphosphoramidocyanidate (tabun, GA), O-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) were characterized for the first time on the basis of their ion mobility and mass spectrometric data. Each compound was headspace sampled for 1 minute with a SPME fiber from a glass surface or from spiked media. Analysis was completed in as little as 1 minute by DESI-IMS-MS^n using the time-aligned parallel (TAP) fragmentation approach. Each chemical warfare agents exhibited a unique ion mobility profiles and up to six MS^n spectra, containing four to eight characteristic ions, including the $[M+H]^+$ ion. Application of the developed approach is anticipated in high sample throughput scenarios where target compound confirmation requires two spectrometric techniques.

A rapid, screening approach was also developed and applied to the analysis of blank and spiked Dacron swab, cardboard and office furniture fabric samples, typical of those that might be collected to support a forensic investigation. Spiked chemical warfare agents were readily detected with S/N ratios in the IMS profile ranging from > 60: 1 to > 7:1. In general, background chemical interference was minimal and the spiked chemical warfare agent was readily identified within a minute using this new method.

Application of this approach is anticipated for high sample throughput scenarios requiring the increased levels of confirmation associated with the use of two spectrometric techniques. Use of the developed methodology is anticipated during forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution or to assess remediation/restoration efforts following an incident.

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Desorption electrospray ionization-mass spectrometric (DESI-MS) analysis has been applied to the direct analysis of chemical warfare agents spiked on a variety of sample media including soils, water, food products and indoor samples that could be collected during a forensic investigation following a chemical incident. Solid phase microextration (SPME) fibers were used in this investigation to sample the headspace above five organophosphorus chemical warfare agents, O-isopropyl methylphosphonofluoridate (sarin, GB), O-pinacolyl methylphosphonofluoridate (soman, GD), O-ethyl N,N-dimethylphosphoramidocyanidate (tabun, GA), O-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX). The exposed SPME fibers were introduced directly into a modified Z-spray electrospray (ESI) source, enabling rapid and safe DESI-MS analysis of the toxic chemical warfare agents. Time-aligned parallel (TAP) fragmentation data, which provides both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n, where n=2 or 3) data for an individual compound, was acquired for the first time for organophosphorus chemical warfare agents. Unique ion mobility profiles and up to six full scanning MS^n spectra, containing the $[M+H]^+$ ion and up to seven diagnostic product ions, were acquired for each chemical warfare agent during DESI-IMS-MS^n analysis of the exposed SPME fiber analysis. A rapid screening approach, based on the developed methodology, was applied to several typical forensic media (Dacron sampling swabs, office furniture fabric and cardboard) spiked with 5 µg of chemical warfare agent. Background interference was minimal and the spiked chemical warfare agents were readily identified within a minute on the basis of the acquired ion mobility and mass spectrometric data. Application of this approach is anticipated for high sample throughput scenarios requiring the increased levels of confirmation associated with the use of two spectrometric techniques.

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Desorption Electrospray Ionization; DESI; Mass Spectrometry; Chemical Warfare Agents; Hydrolysis Products Ion Mobility Spectrometry; Time-Aligned Parallel Fragmentation Data

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